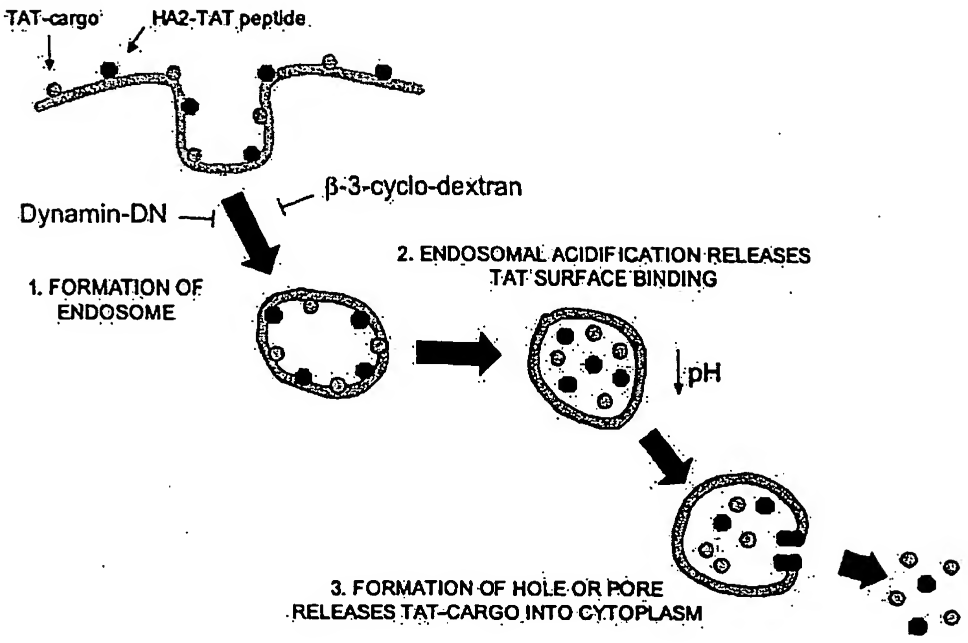

TAT-cargo
HA2-TAT peptide
β-3-cyclo-dextran
Dynamin-DN
1. FORMATION OF ENDOSOME
2. ENDOSOMAL ACIDIFICATION RELEASES TAT SURFACE BINDING
pH
3. FORMATION OF HOLE OR PORE RELEASES TAT-CARGO INTO CYTOPLASM

FIG. 1

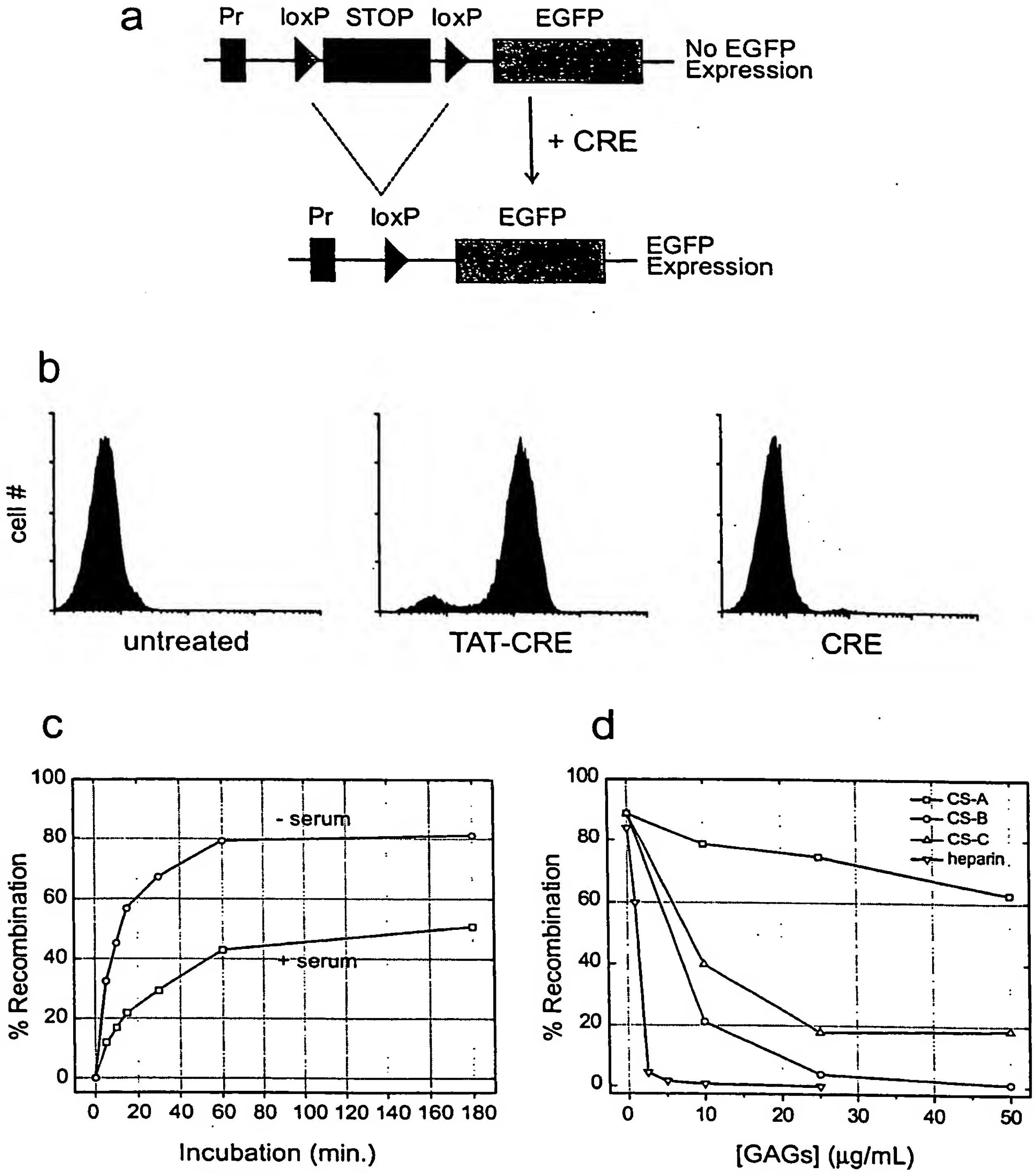

a
Pr
loxP
STOP
loxP
EGFP
No EGFP Expression
+ CRE
Pr
loxP
EGFP
EGFP Expression
b
cell #
untreated
TAT-CRE
CRE
c
% Recombination
100
80
60
40
20
0
- serum
+ serum
0 20 40 60 80 100 120 140 160 180
Incubation (min.)
d
% Recombination
100
80
60
40
20
0
CS-A
CS-B
CS-C
heparin
0 10 20 30 40 50
[GAGs] (µg/mL)

FIG. 2A-D

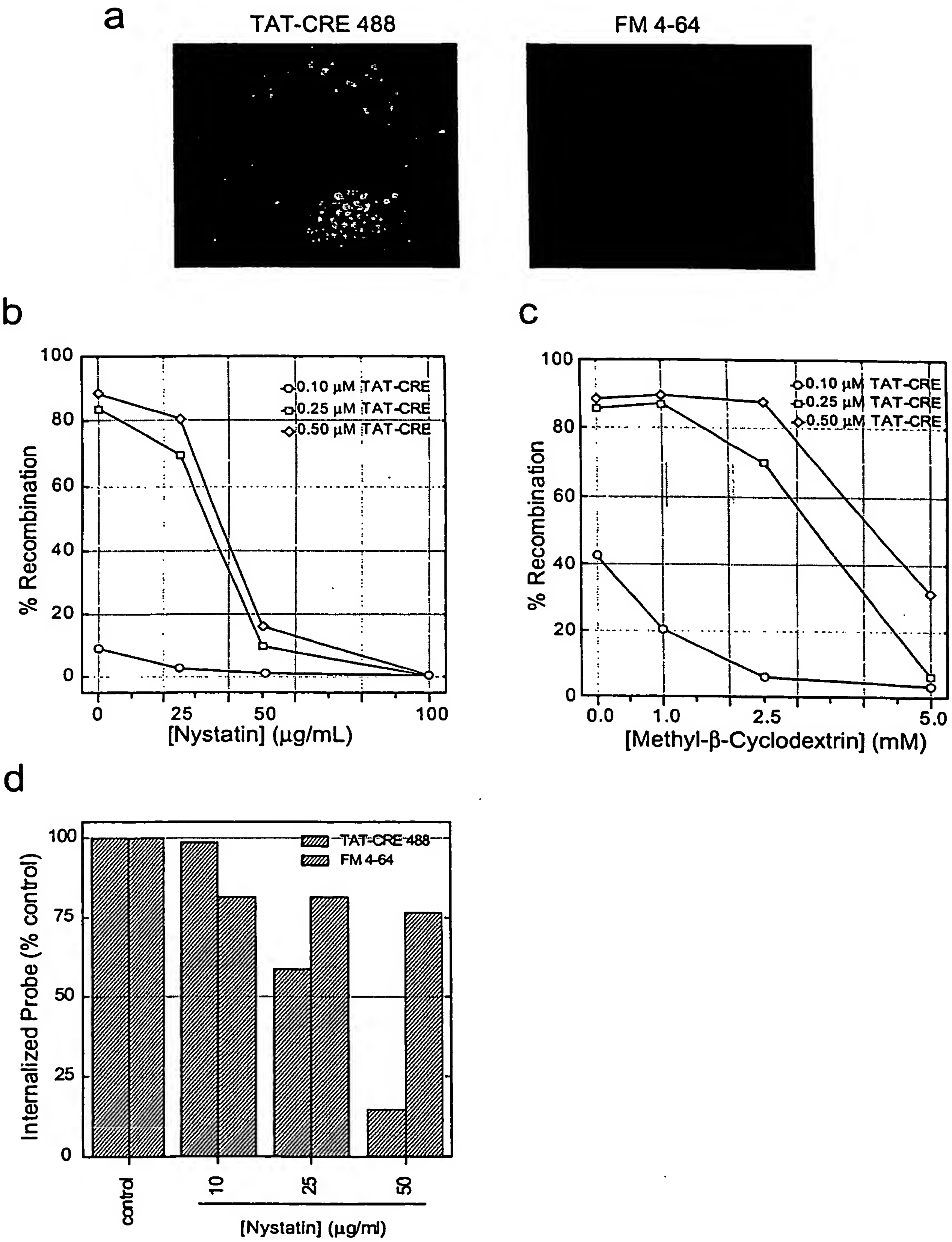

a
TAT-CRE 488
FM 4-64
b
% Recombination
100
80
60
40
20
0
0
25
50
100
[Nystatin] (µg/mL)
0.10 µM TAT-CRE
0.25 µM TAT-CRE
0.50 µM TAT-CRE
c
% Recombination
100
80
60
40
20
0
0.0
1.0
2.5
5.0
[Methyl-β-Cyclodextrin] (mM)
0.10 µM TAT-CRE
0.25 µM TAT-CRE
0.50 µM TAT-CRE
d
Internalized Probe (% control)
100
75
50
25
0
TAT-CRE 488
FM 4-64
control
10
25
50
[Nystatin] (µg/ml)

FIG. 3A-D

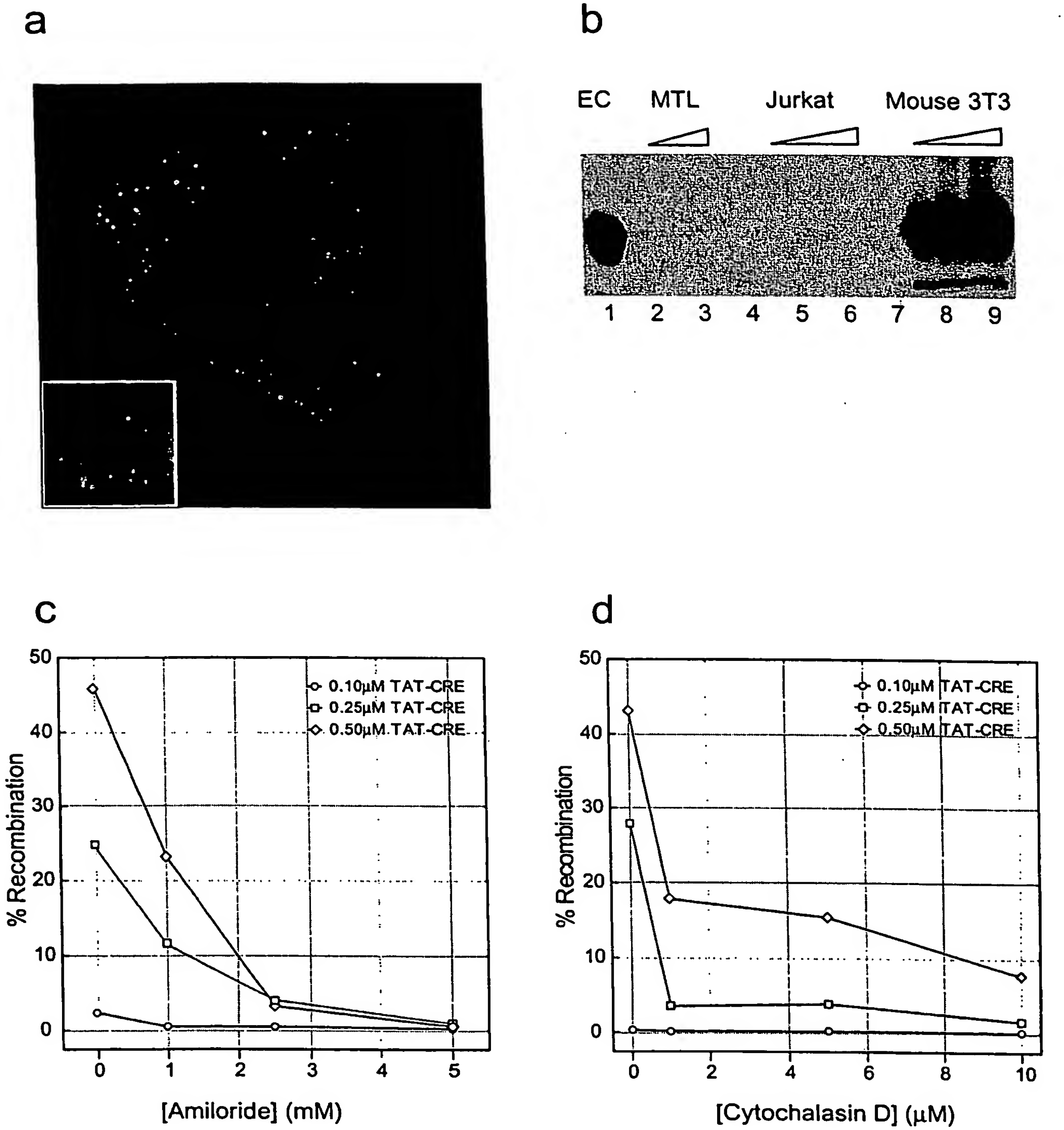

a
b
EC
MTL
Jurkat
Mouse 3T3
1
2
3
4
5
6
7
8
9
c
0.10μM TAT-CRE
0.25μM TAT-CRE
0.50μM TAT-CRE
% Recombination
50
40
30
20
10
0
0
1
2
3
4
5
[Amiloride] (mM)
d
0.10μM TAT-CRE
0.25μM TAT-CRE
0.50μM TAT-CRE
% Recombination
50
40
30
20
10
0
0
2
4
6
8
10
[Cytochalasin D] (μM)

FIG. 4A-D

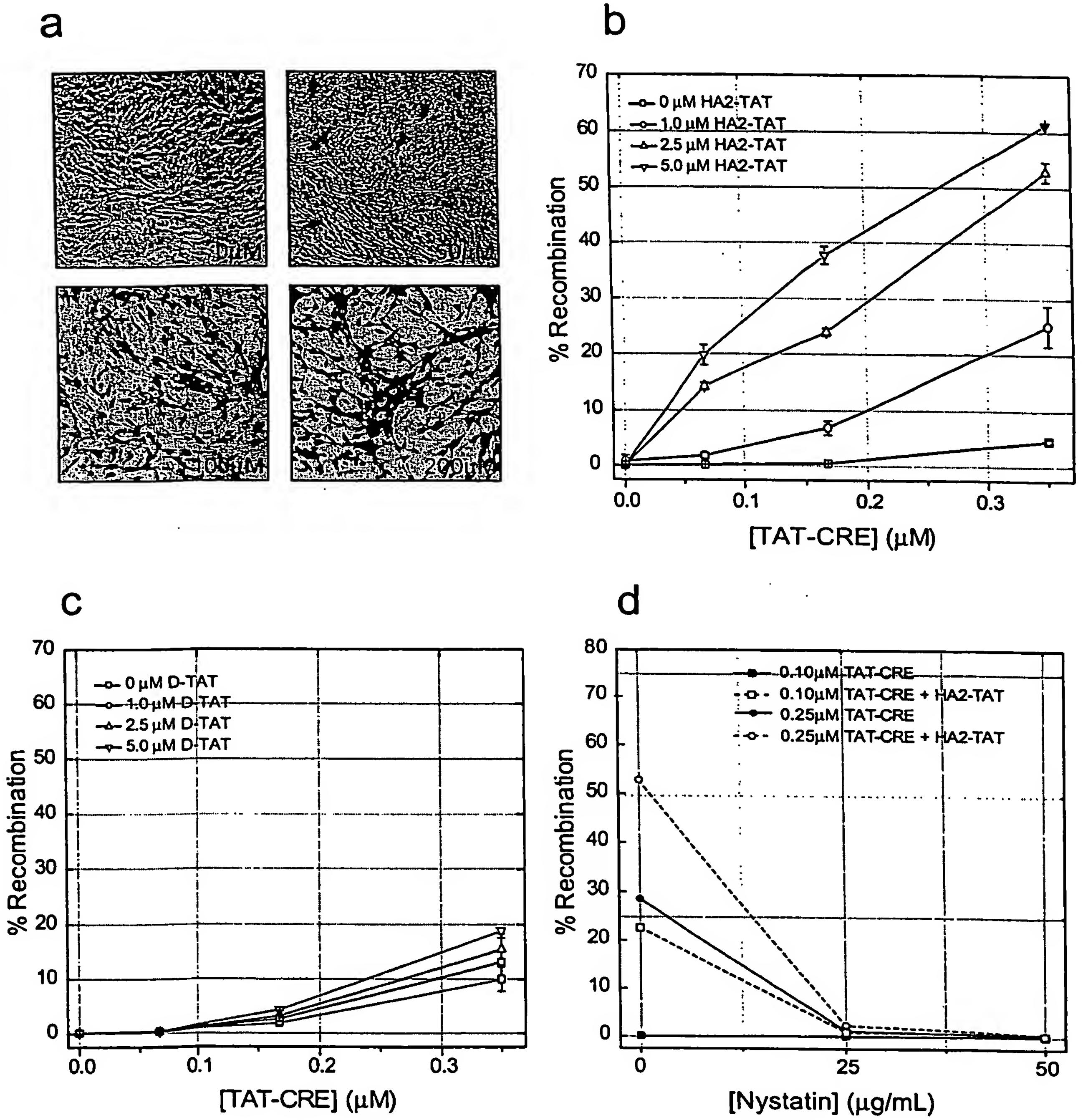
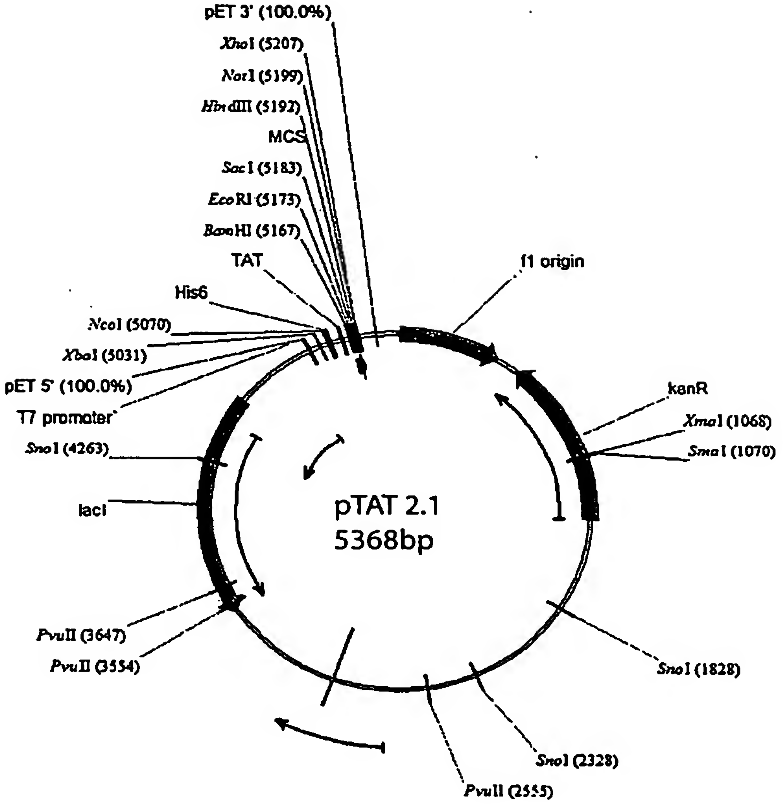
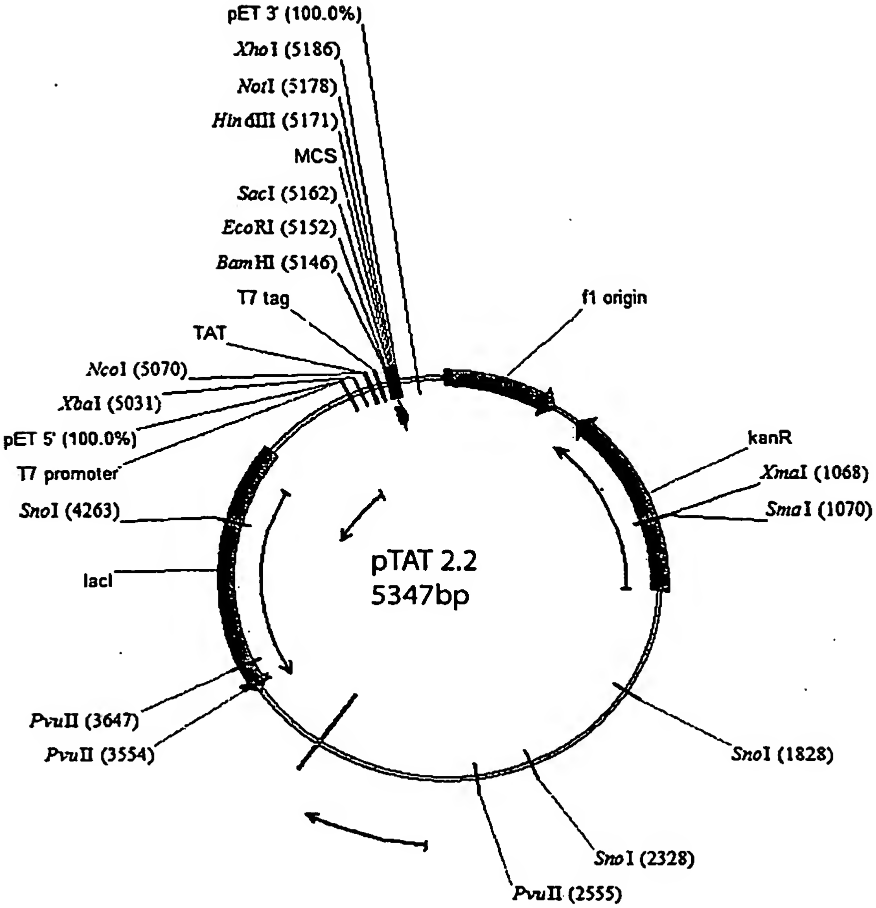

a
0 µM
50 µM
100 µM
200 µM
b
0 µM HA2-TAT
1.0 µM HA2-TAT
2.5 µM HA2-TAT
5.0 µM HA2-TAT
% Recombination
70
60
50
40
30
20
10
0
0.0
0.1
0.2
0.3
[TAT-CRE] (µM)
c
0 µM D-TAT
1.0 µM D-TAT
2.5 µM D-TAT
5.0 µM D-TAT
% Recombination
70
60
50
40
30
20
10
0
0.0
0.1
0.2
0.3
[TAT-CRE] (µM)
d
0.10µM TAT-CRE
0.10µM TAT-CRE + HA2-TAT
0.25µM TAT-CRE
0.25µM TAT-CRE + HA2-TAT
% Recombination
80
70
60
50
40
30
20
10
0
0
25
50
[Nystatin] (µg/mL)

FIG. 5A-D



```
                                               T7 promoter'                                 XbaI
                                              pET 5' 100.0%
4951  GCGTAGAGGA TCGAGATCTC GATCCCGCGA AATTAATACG ACTCACTATA GGGGAATTGT GAGCGGATAA CAATTCCCCT CTAGAAATAA
      CGCATCTCCT AGCTCTAGAG CTAGGGCGCT TTAATTATGC TGAGTGATAT CCCCTTAACA CTCGCCTATT GTTAAGGGGA GATCTTTATT
.......................................................................................................
                                   NcoI            His6
 +1                                 M  G  S  S  H  H  H  H  H  H  S  S  G  L  V  P  R  G  S  H
5041  TTTTGTTTAA CTTTAAGAAG GAGATATACC ATGGGCAGCA GCCATCATCA TCATCATCAC AGCAGCGGCC TGGTGCCGCG CGGCAGCCAT
      AAAACAAATT GAAATTCTTC CTCTATATGG TACCCGTCGT CGGTAGTAGT AGTAGTAGTG TCGTCGCCGG ACCACGGCGC GCCGTCGGTA
.......................................................................................................
                     TAT                                           MCS
                                                 EcoRI                      NotI
                                           BamHI         SacI         HindIII        XhoI
 +1   M  R  K  K  R  R  Q  R  R  R  G  S  D  P  N  S  S  S  V  D  K  L  A  A  A  L  E  H  H  H
5131  ATGAGGAAGA AGCGGAGACA GCGACGAAGA GGCTCGGATC CGAATTCGAG CTCCGTCGAC AAGCTTGCGG CCGCACTCGA GCACCACCAC
      TACTCCTTCT TCGCCTCTGT CGCTGCTTCT CCGAGCCTAG GCTTAAGCTC GAGGCAGCTG TTCGAACGCC GGCGTGAGCT CGTGGTGGTG
.......................................................................................................
 +1   H  H  H
5221  CACCACCACT GAGATCCGGC TGCTAACAAA GCCCGAAAGG AAGCTGAGTT GGCTGCTGCC ACCGCTGAGC AATAACTAGC ATAACCCCTT
      GTGGTGGTGA CTCTAGGCCG ACGATTGTTT CGGGCTTTCC TTCGACTCAA CCGACGACGG TGGCGACTCG TTATTGATCG TATTGGGGAA
                                                                         pET 3' 100.0%
.......................................................................................................
5311  GGGGCCTCTA AACGGGTCTT GAGGGGTTTT TTGCTGAAAG GAGGAACTAT ATCCGGAT
      CCCCGGAGAT TTGCCCAGAA CTCCCCAAAA AACGACTTTC CTCCTTGATA TAGGCCTA
.......................................................................................................
```

FIG. 6



```
                                           T7 promoter'                                          XbaI
                                            pET 5' 100.0%
4951  GCGTAGAGGA TCGAGATCTC GATCCCGCGA AATTAATACG ACTCACTATA GGGGAATTGT GAGCGGATAA CAATTCCCCT CTAGAAATAA
      CGCATCTCCT AGCTCTAGAG CTAGGGCGCT TTAATTATGC TGAGTGATAT CCCCTTAACA CTCGCCTATT GTTAAGGGGA GATCTTTATT

                                         NcoI                  TAT                          T7 tag
+1                                        M  G  R  K  K  R  R  Q  R  R  R  G  H  M  A  S  M  T  G  G
5041  TTTTGTTTAA CTTTAAGAAG GAGATATACC ATGGGCAGGA AGAAGCGGAG ACAGCGACGA AGAGGCCATA TGGCTAGCAT GACTGGTGGA
      AAAACAAATT GAAATTCTTC CTCTATATGG TACCCGTCCT TCTTCGCCTC TGTCGCTGCT TCTCCGGTAT ACCGATCGTA CTGACCACCT

       T7 tag                          MCS
                              EcoRI                                    NotI
                    BamHI                SacI            HindIII                  XhoI
+1     Q  Q  M  G  R  D  P  N  S  S  S  V  D  K  L  A  A  A  L  E  H  H  H  H  H  H  *
5131  CAGCAAATGG GTCGGGATCC GAATTCGAGC TCCGTCGACA AGCTTGCGGC CGCACTCGAG CACCACCACC ACCACCACTG AGATCCGGCT
      GTCGTTTACC CAGCCCTAGG CTTAAGCTCG AGGCAGCTGT TCGAACGCCG GCGTGAGCTC GTGGTGGTGG TGGTGGTGAC TCTAGGCCGA

5221  GCTAACAAAG CCCGAAAGGA AGCTGAGTTG GCTGCTGCCA CCGCTGAGCA ATAACTAGCA TAACCCCTTG GGGCCTCTAA ACGGGTCTTG
      CGATTGTTTC GGGCTTTCCT TCGACTCAAC CGACGACGGT GGCGACTCGT TATTGATCGT ATTGGGGAAC CCCGGAGATT TGCCCAGAAC
                                                  pET 3' 100.0%
```

FIG. 7

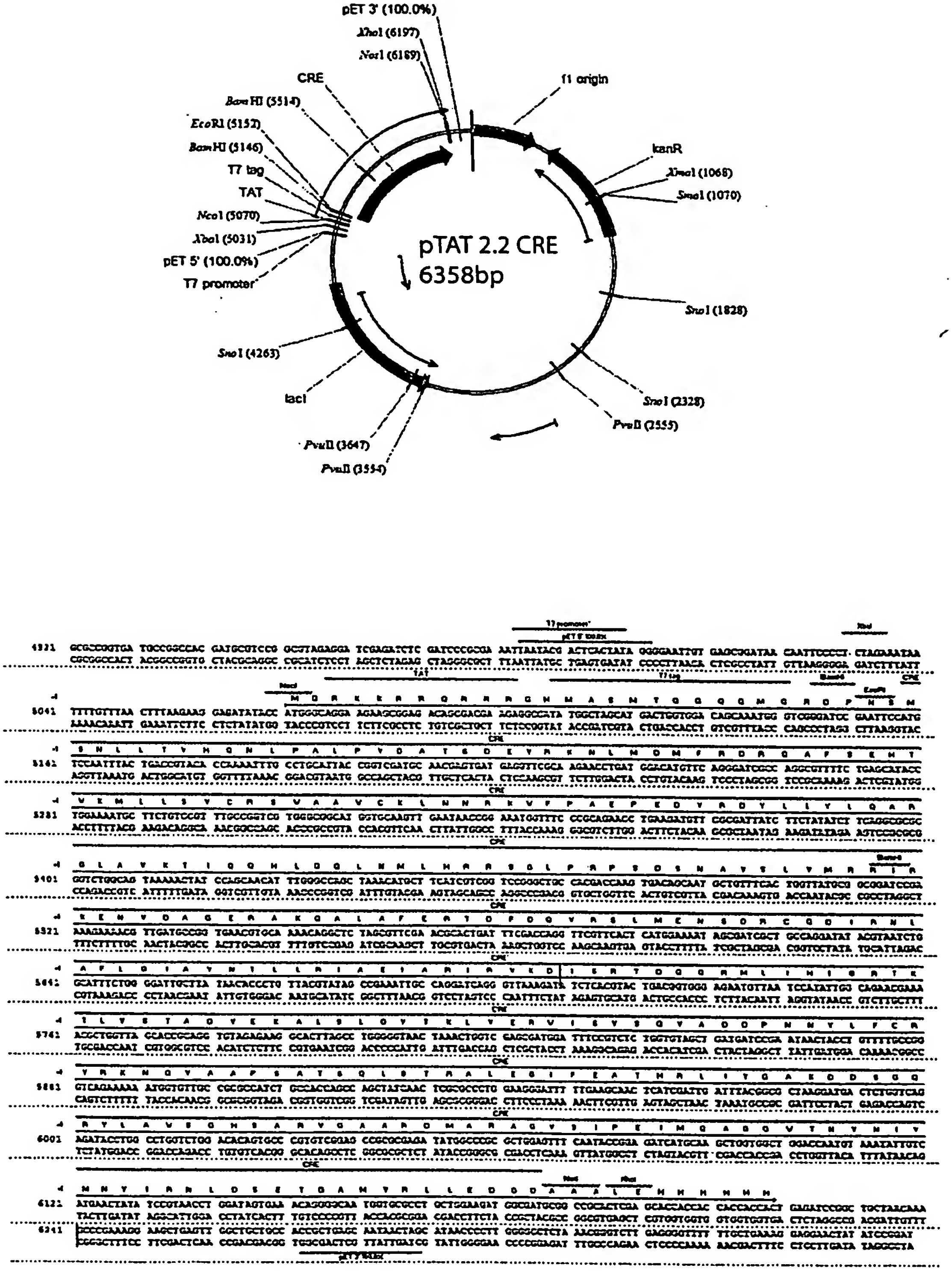

pET 3' (100.0%)
XhoI (6197)
NotI (6189)
f1 origin
CRE
BamHI (5514)
EcoRI (5152)
BamHI (5146)
T7 tag
TAT
NcoI (5070)
XbaI (5031)
pET 5' (100.0%)
T7 promoter
kanR
XmaI (1068)
SmaI (1070)
pTAT 2.2 CRE
6358bp
SnoI (1828)
SnoI (4263)
lacI
SnoI (2328)
PvuII (2555)
PvuII (3647)
PvuII (3554)
T7 promoter
pET 5' (100.0%)
XbaI
TAT
T7 tag
NcoI
BamHI
EcoRI
CRE
NotI
XhoI
pET 3' (100.0%)
4921
5041
5161
5281
5401
5521
5641
5761
5881
6001
6121
6241

FIG. 8